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Nutritional ingredients from spent brewer's yeast obtained by hydrolysis and selective membrane filtration integrated in a pilot process

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ABSTRACT

Spent brewer's yeast is a natural surplus from brewing industry. In order to up-grade this by-product, isolation of compounds has been tentatively assessed. The main objective of this work focuses on the use of ultrafiltration and nanofiltration pilot system for recovering cell compounds. Initially, yeast was autolyzed and ultrafiltered with a 10 kDa cut-off, and the two fractions obtained were hydrolyzed with Cynara cardunculus extract and nanofiltered with 3 kDa cut-off. Four fractions with different molecular weights were obtained, with protein and sugar contents ranging between 30–69% and 20–48%, respectively. Sodium and potassium were the major minerals present, whereas glutamine, glutamic acid and alanine, the most representative free amino acids. Peptide profile showed peptides with hydrophilic and hydrophobic characteristics, usually associated with biological activities, including antihypertensive and antioxidant. Thus, based on their compositions, all fractions show technological and biological potential, and can be used as nutritional ingredients in food and feed.

1. Introduction

Beer manufacture involves production of several residues and by-products e.g., spent grains, hops and yeasts (Mussatto, 2009). Brewer's yeast is the second largest by-product originated by the food industry, and most of this is sold as animal feed at low price or has to be disposed as waste. Therefore it merits considerable attention, not only for the large amounts produced, but also due to its valuable nutritional composition. Residual brewing yeast is predominantly composed by proteins (35–60% dry basis) that include all the essential amino acids and have high biological value (referring to the amount of essential amino acids in its structure) (Chae et al., 2001) thus being an excellent source of high-quality protein, comparable in value with soy protein (Otero et al., 2000). The second highest compound is carbohydrates that represent 35–45% of dry basis. In addition, this by-product has other substances biologically important, such as, minerals (5–7.5% of which, Ca, P, K, Mg, Fe, among others) lipids, B vitamins and enzymes (dos Santos Mathias et al., 2014).

Yeasts have been traditionally used in fermentation processes, as food flavoring and enrichment ingredients as yeast extract and autolysate (Pacheco et al., 1997), and are generally recognized as safe - GRAS (Briggs et al., 2004). Due to this, for inactivated yeast derivatives, new applications have been explored as nutritive complements and ingredients for formulations in food industry (Chae et al., 2001; Dikit et al., 2010).

This increasing interest in food by-products led to the development of novel bioprocessing technologies for isolation of bioactive substances to be used as functional foods and nutraceuticals. Improvement of these functional ingredients, involves certain biotransformation processes through enzyme-mediated hydrolysis in combination with membrane techniques for fractionation of peptides hydrolysate. Porous membrane filtration is employed as a physical barrier in order to separate particles with different characteristics, based on size and shape, using pressures and membranes specifically designed for the process, with different pore

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diameters (Malik et al., 2013). Although, it exist different methods of membrane filtration - reverse osmosis, nanofiltration, ultrafiltration and microfiltration, in ascending order relative to the diameter of the pores, they are all intended for separation or concentration of substances (Pouliot et al., 2014).

Several studies relate the application of this technology to the production of food ingredients and nutraceuticals. Dairy by-products, lead the utilization of this technology in the production of purified and hydrolyzed proteins, lactose and salts (Lieske and Konrad, 1996; Saxena et al., 2009; Tavares et al., 2012), but other food industries including canned fish, meat, cereals, among others have been using this technology (Samaranayaka and Li-Chan, 2011). Thereby, membrane-based process can provide the required product quality, purity, yield and throughput with low cost and environmental sustainability. All these assumptions lead to the main objective of this work, the use of selective membranes, in pilot system, to transform the spent brewer's yeast in hydrolyzed fractions with different molecular weights and with improved chemical and nutritional richness.

2. Material and methods

2.1. Spent brewer's yeast

Spent brewer's yeast was obtained as a byproduct of beer production and it was kindly provided by UNICER, Porto, Portugal. Each batch of spent brewer's yeast processed in pilot process was initially submitted to autolysis performed at 70 °C for 4 h in a 100 L double-walled, steam-supplied vat heated with burning gas with control for stirring rate and temperature.

2.2. Development of pilot process

Spent brewer's yeast autolysate (stored and transported at refrigerated temperature) was submitted to a sequence of selective filtration processes (Fig. 1), combined with enzymatic hydrolysis to obtain different peptide extracts, using pilot-scale equipment (Proquiga, Spain). As described in Fig. 1, 100 L of spent brewer's yeast autolysate was factionated in an ultrafiltration batch system at 45–50 °C, with an organic membrane Hydranautics model (Dairy 10k 3838-30) with 7.4 m^2 filtration area and 10 kDa cut-off. Thereafter, protein retentate (PR) (molecular weight (MW) > 10 kDa) and protein permeate (PP) (MW < 10 kDa) were submitted to hydrolysis at optimal conditions previously determined (data not shown): 4% (v/v) of *Cynara cardunculus* extract (Formulab, Maia, Portugal), for 4 h at 55 °C and pH adjusted to 5.2 with lactic acid (Sigma – Aldrich, St. Louis MO, USA). After hydrolysis, each hydrolyzed fraction (protein retentate and permeate) was then nanofiltred at 45–55 °C using an organic membrane PTI Advanced Filtration (model NF 3838/30-FF), area 6, 9 m^2 with 3 kDa cut-off.

Four fractions were obtained (i) Protein Retentate Hydrolyzed >3 kDa (PRH >3 kDa); (ii) Protein Retentate Hydrolyzed <3 kDa (PRH <3 kDa); (iii) Protein Permeate Hydrolyzed >3 kDa (PPH >3 kDa) and (iv) Protein Permeate Hydrolyzed <3 kDa (PPH <3 kDa). Each of these fractions was concentrated by reverse osmosis (concentration rate approximately 40 times), frozen at −80 °C and then freeze dried and stored under vacuum at ambient temperature, protected from light.

2.3. Nutritional composition

Protein content was determined according AOAC procedures (Nx5.8) (Horwitz et al., 2010) using a Kjeltec system 1002 distilling unit (Tecator; Höganäs, Sweden).

Total sugars were determined by colorimetric method as described by Dubois et al. (1956), using glucose (Sigma – Aldrich, St. Louis MO, USA) as standard. Ashes were determined by heating for 5 h in a muffle at 525 °C (AOAC, 1995).

Mineral concentration was carried out by optical emission spectrometer Model Optima 7000 DV ™ ICP-OES (Dual View, PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) with radial configuration as described by Chatelain et al. (2014). Free amino acids content of each fraction was performed by pre-column derivatization with orthophthalaldehyde (OPA) methodology. Isoindole-type fluorescent derivatives were formed in an alkaline solution (borate buffer pH 10.4) from OPA, 2-sulfanylethanol and the primary amine group of the amino acid. The derivatives were

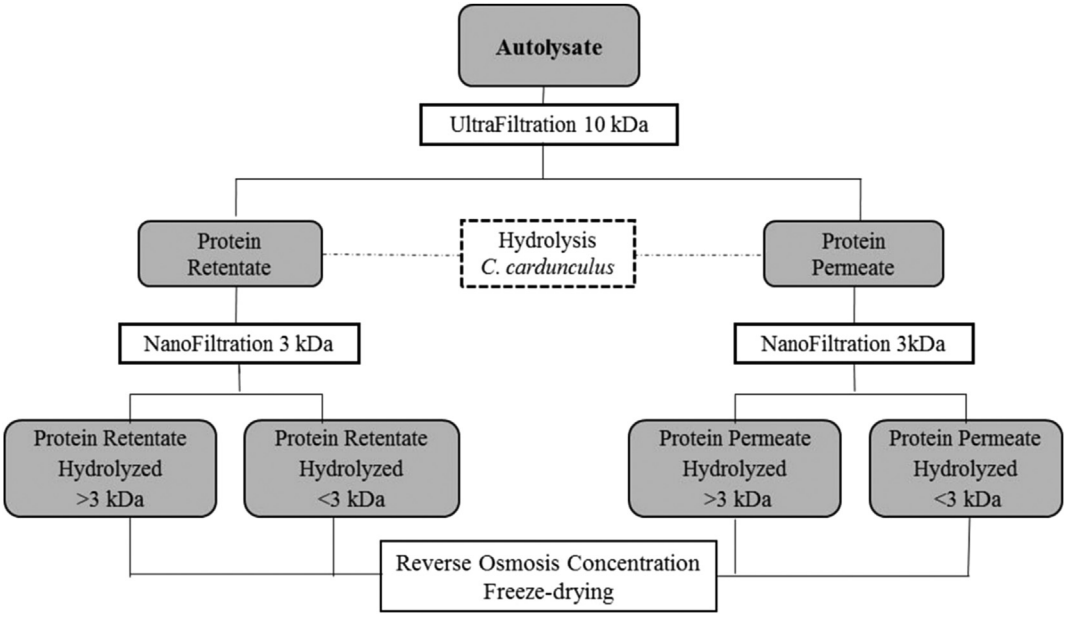

Fig. 1. Diagram of pilot scale for filtration of spent brewer's yeast autolysate.

separated by HPLC (Beckman coulter, California USA) coupled to a fluorescence detector (Waters, Milford. MA, USA) according to the procedure of Proestos et al. (2008). 100 μL of each sample, at concentration 10 mg mL^{-1}, was derivatized according described method and injection volume of derivatives was 20 μL. All analysis were made in triplicate, and quantified using a calibration curve built with amino acids pure standards (Sigma – Aldrich, St. Louis MO, USA) and expressed as g 100 g^{-1} of protein content.

2.4. Peptide profile

Peptide profiles of all <3 kDa cutoff fractions were analyzed by reverse phase - high performance liquid chromatography (RP-HPLC) using a Beckman Coulter unit equipped with Karat32 software and a C18 column (COSMOSIL 5C18-AR-II), held at room temperature (20 °C). Separation was carried out using two eluents: eluent A: 0.1% trifluoroacetic acid (TFA) in ultrapure water (v/v), and eluent B: 0.1% TFA in acetonitrile (v/v) and gradient elution as follows: 0–20 min 0% of B; 20–40 min: gradient B increase until 100% and finally 40–50 min B decrease until 0%. The flow rate was 0.8 mL min^{-1} and the detection was carried out at 220 nm (Beckman Diode Array 168). For the chromatographic analysis, lyophilized samples were resuspended into ultrapure water (5 mg mL^{-1}) and volume injected was 20 μL.

3. Results and discussion

3.1. Pilot scale

Semi-industrial filtration process was optimized, as shown in Fig. 1, and the main objective was obtaining fractions with nutritive value, with special emphasis in hydrolyzed concentrates. As mentioned above, membrane techniques are extremely important for industry, since they are easily applicable on a large scale processing and are also economically advantageous when compared with other techniques (Tavares et al., 2012). Predefined amount of spent brewer's yeast (100 L), previously autolyzed (70 °C for 4 h), and containing approximately 49% protein, was ultrafiltered for concentration of proteins and peptides using a cut-off of 10 kDa. After ultrafiltration, two fractions – 50 L of Protein Retentate (PR) and 50 L of Protein Permeate (PP) were hydrolyzed with 4% of enzymatic extract from vegetable curd, *Cynara cardunculus*. After hydrolysis, each hydrolyzed fraction was nanofiltered with 3 kDa cut-off, allowing a potential protein, peptides, amino acids recovering and other added value compounds like minerals, oligopeptides and polysaccharides, which can offers some selectivity of the final extract profile. Furthermore, authors have associated smaller peptides (<3 kDa) with biological activities, e.g. antihypertensive (Tavares et al., 2011), antioxidant (Sarmadi and Ismail, 2010), antimicrobial (Bamdad et al., 2015). All resultant fractions were concentrated by reverse osmosis to reduce water and dehydrated by lyophilization, thus obtaining a free flowing powder with good appearance and pleasant odor. Four added value fractions with different molecular weight were obtained: (i) PRH >3 kDa; (ii) PRH <3 kDa; (iii) PPH >3 kDa and (iv) PPH <3 kDa.

Table 1
Mass balance for semi-industrial process from 100 L of spent brewer's yeast autolysate.

Fraction	Total mass (g)
PRH >3 kDa	650
PRH <3 kDa	125
PPH >3 kDa	125
PPH <3 kDa	70

Mass balance of the fractions obtained from spent brewer's yeast is depicted in Table 1. For 100 L of autolysate, it was obtained approximately 1 kg of dry matter. PRH >3 kDa fraction had the highest amount of dry matter, since this fraction includes a large amount of polysaccharides from yeast cell wall. From this fraction it is possible to obtain rich portions of betaglucans and another's polysaccharides with high biological interest (Thammakiti et al., 2004).

Several authors report ultrafiltration processes to produce peptides and protein extracts from different food sources - whey (Tavares et al., 2012); soy bean (Moure et al., 2006); lupin (D'Agostina et al., 2006), alfalfa (Firdaous et al., 2009), among others. However for spent brewer's yeast, only Huang (2012) relate an optimization study for separation of protein and polysaccharide by ultrafiltration processes. Therefore, this study represents the first approach to produce added-value extracts from brewer's spent yeast combining autolysis, enzymatic hydrolysis and selective membrane filtration.

3.2. Nutritional composition

The physicochemical composition of each fraction was different as presented in Table 2 regarding protein, sugars and minerals content. These fractions are an important source of protein, ranging between 35 and 70% dry basis, being higher for high MW fractions (PRH >3 kDa and PPH >3 kDa), however these values are within protein recovery intervals described in other studies (Li et al., 2009; Otero et al., 2000; Yamada and Sgarbieri, 2005). The carbohydrate content is also important; being higher in lower molecular weight fractions (PRH <3 kDa and PPH <3 kDa), due to the presence of simple sugars liberated in autolysis. The disruption of cell wall, which is composed mainly of glucans and mannans (Jaehrig et al., 2008), leads to an increase of the accessibility of polysaccharides with biological value (betaglucans) and small fragments of saccharides polymers – oligosaccharides, which can be separated by ultrafiltration and may be present in PRH >3 kDa and PPH >3 kDa fractions, respectively.

As expected, fractions have different amounts of minerals. Fractions with lower MW < 3 kDa present a higher percentage of minerals, namely sodium (Na) and potassium (K) (Table 2). Recently, it have been proposal to replace the absolute potassium and sodium with recommended sodium-potassium ratios (Drewnowski et al., 2012). Epidemiological studies (Cook et al., 2009; Perez and Chang, 2014; Whelton, 2014) indicates a relationship between blood pressure and sodium/potassium ratios is more readily demonstrable within population than a relationship with either sodium or potassium alone. They relate, that lowering dietary sodium intake, while increasing potassium consumption, at the population level might reduce the incidence of cardiovascular

Table 2
Protein, sugar and mineral content (% w/w, dry basis) of each yeast fractions obtained in semi-industrial system.

	PRH >3 kDa	PRH <3 kDa	PPH >3 kDa	PPH <3 kDa
Protein	69 ± 0.12	30 ± 0.13	40 ± 0.90	35 ± 0.10
Sugars	27 ± 0.10	40 ± 0.11	48 ± 0.10	42 ± 0.10
Ash	3 ± 0.09	22 ± 0.85	8 ± 0.65	14 ± 0.45
Minerals				
P	0.963 ± 0.05	1.03 ± 0.06	1.77 ± 0.01	1.37 ± 0.14
Mg	0.143 ± 0.01	0.68 ± 0.04	0.19 ± 0.01	< LOD
Ca	0.367 ± 0.03	0.40 ± 0.05	< LOD	< LOD
Na	0.478 ± 0.03	11.81 ± 0.32	2.77 ± 0.04	5.52 ± 0.41
K	0.679 ± 0.03	7.95 ± 0.19	3.21 ± 0.07	6.62 ± 0.55

Results were expressed as mean ± standard deviation.
< LOD – Low limit of detection.

disease. In this process PPH >3 kDa and PPH <3 kDa fractions, possess lowers ratios for Na/K, since potassium concentration is higher; this attests the latest reports that ensure the combined use of sodium and potassium, suggesting they can be used as salt supplements for individuals in the prevention of cardiovascular disease and high blood pressure.

Moreover, food industry is trying to reduce NaCl in foods by replacing sodium with other substances. Potassium, used on chloride form, is the most common salt substitute used in low or reduced salt/sodium foods (Desmond, 2006). Potassium chloride has physical properties similar to sodium salt and has approximately 80% of the capacity of salting but has bitter taste (Cruz et al., 2011). To avoid this problem, some mixtures with other minerals have been commercialized (e.g. Pansalt®, Lo salt®, Morton Lite Salt®, among others) in order to mask the undesired taste in foods. In this study for fractions PPH <3 KDa and PPH >3 KDa fractions, the potassium levels exceeds sodium content, as well possess a mixture of phosphorus, magnesium and calcium, which is advantageous and in accordance with commercial salt substitutes, suggesting the use of these extracts as natural source of minerals to be used as taste and health promoters.

On the other hand, despite the sodium levels be present at high levels for PRH <3 kDa fraction, the consumption of these fractions as a food additive does not exceed the recommended daily salt content (5 g – WHO (WHO, 2009)), if consumed in controlled daily doses. Sodium is present in all fractions and could be in different chemical forms, including monosodium salt or monosodium glutamate, a taste enhancer and a reducer of bitter notes in the food (Jinap and Hajeb, 2010). Thus, these fractions can be a natural replacer of monosodium glutamate, in order to decrease the salt content of foodstuffs and also for the possibility to express the label "natural additive" as defined by FDA - Food and Drug Administration (Lavine, 2007). Based on these evidences, we suggest that PRH <3 kDa; PPH >3 kDa and PPH <3 kDa fractions can be used as common salt substitute or/and added to food in order to enrich or fortify mineral content.

Quantitative determination of free amino acid was performed by HPLC and concentration is shown in Table 3. Eight free amino acids are present in relevant contents except for PRH >3 kDa, where only three amino acids are at vestigial concentrations. This is in accordance with the process, since free amino acids have low MW, so they are not retained by the membranes during the initial ultrafiltration. Glutamic acid and glutamine contents are high and they are closely related in a chemical sense. Even though they are not essential amino acids being synthesized by the body, but food industries makes use of their flavor enhancing properties, and, for this reason, are widely used, particularly in the form of the monosodium salt. Monosodium glutamate (MSG) provides the typical aroma "umami", recognized as the fifth basic taste, very similar to "meat aroma" (Populin et al., 2007). Thus the yeast extract of the fractions can be used as a "hidden ingredient" of MSG, having a high demand for food companies. Alanine concentration is also high in particular in PRH <3 KDa, and is an important amino acid in muscle synthesis, which is widely used as athletic performance supplement (del Favero et al., 2012). Arginine is present in considerable amounts in fractions with low MW, especially for PRH <3 kDa fraction. Dietary arginine supplementation may represent a potentially useful strategy for the management of diabetes (Kohli et al., 2004) and immune stimulatory effects (May et al., 2002). Arginine and alanine are stable amino acids, which are not destroyed by high temperature and high pressure. Aspartic acid, which has several biological activities in humans and animals, is only present in PPH >3 kDa fraction and, technologically is a key raw amino acid for the synthesis of artificial high intensity sweeteners such as aspartame (Kroger et al., 2006). Aspartic acid is also essential to the process of chelating minerals improving their assimilation, digestion, and utilization, as is the case of calcium and potassium, among others (Sajadi, 2010). Although exists more amino acids in PRH<3 kDa fraction than in PPH<3 kDa, this occurs due more amino acid release during protein hydrolysis being more concentrated in this fraction, fact consistent throughout different batches process. Thus, the diversity of free amino acids present in the different fractions are known to exert a major influence on flavor and also makes them of great value from a nutritional, chemical and biochemical point of view, allowing them to be used as amino acid supplements in animal and human diets.

3.3. RP-HPLC peptide profile

Scientific interest has focused on bioactive peptides derived from food proteins, and physiologically effects are related to low MW peptides (Gómez-Ruiz et al., 2007). Thus, in view of hydrolysis realized in this process, low MW fractions were studied by RP-HPLC. This technique, using a C18 column, allows us a preliminary study of the peptide composition by adsorption analysis of peptides to a hydrophobic stationary matrix (Perez Espitia et al., 2012) and predict their potential bioactivity according to the composition. The final peptide profiles of both yeast hydrolysates PRH and PPH <3 kDa cutoff, are depicted in Fig. 2. The chromatograms show a major elution of molecules between 0 and 24 min, at which stage the mobile phase is 100% water, or hydrophilic. Thus we can predict that those fractions correspond to peptides that have in its constitution hydrophilic amino acids such as aspartic acid, glutamic acid, histidine, lysine, glutamine, asparagine threonine, arginine, and serine, relating them with some biological activities such as antimicrobial (Papo and Shai, 2003) and opioid (Kitts and Weiler, 2003). Regarding PRH <3 kDa fraction, it appears a mass between 27 and 35 min, corresponding to a stage in which,

Table 3
Free amino acid composition of each yeast fraction.

g/100 g of protein content	PRH >3 kDa	PRH <3 kDa	PPH >3 kDa	PPH <3 kDa
Glutamic acid	0.63 ± 0.02	5.57 ± 0.04	15.0 ± 0.09	7.56 ± 0.10
Aspartic acid	ND	ND	4.08 ± 0.05	ND
Glutamine	ND	17.99 ± 0.12	7.65 ± 0.11	8.17 ± 0.02
Threonine[a]	0.23 ± 0.09	ND	ND	ND
Arginine	0.28 ± 0.07	11.25 ± 0.10	4.68 ± 0.08	5.34 ± 0.22
Alanine	ND	26.63 ± 0.08	11 ± 0.09	17.5 ± 0.07
Tirosine	ND	0.87 ± 0.03	ND	0.39 ± 0.04
Valine[a]	ND	0.78 ± 0.06	ND	0.69 ± 0.09
Total	1.14	63.09	42.41	39.65

Results were expressed as mean ± standard deviation.
ND – Not detected.
[a] Essential amino acid.

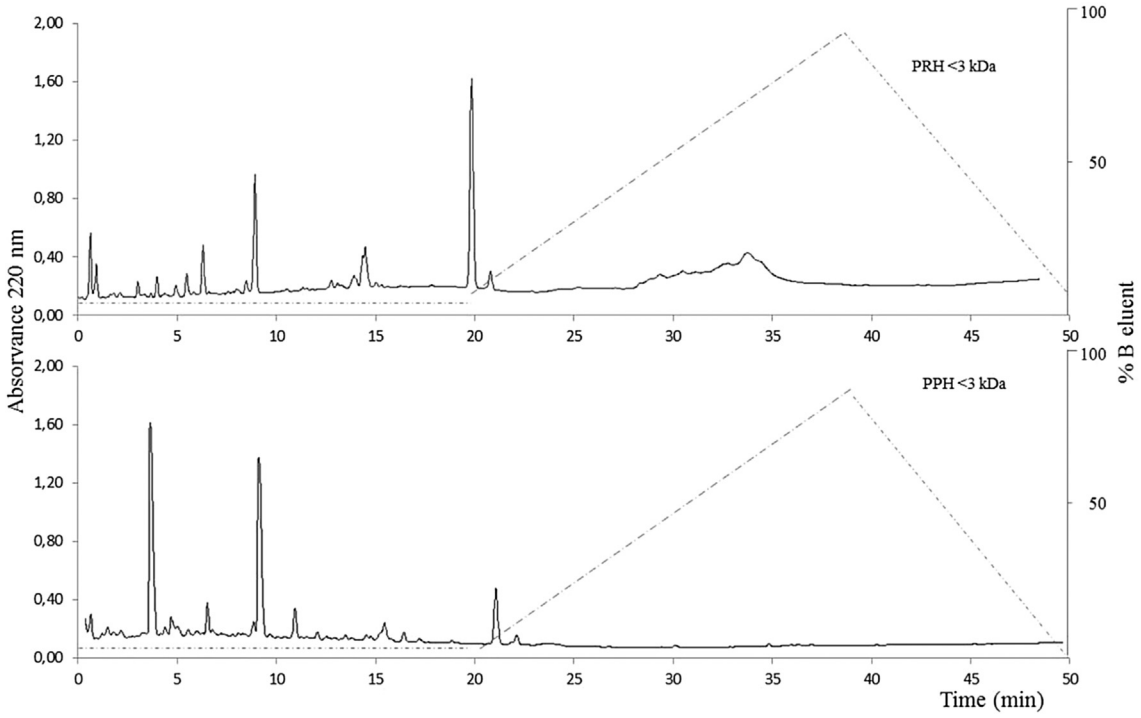

Fig. 2. RP-HPLC chromatograms for protein retentate hydrolyzed <3 kDa (PRH <3 kDa) and protein permeate hydrolyzed <3 kDa (PPH <3 kDa), as well as percentage of B eluent.

exists a mixture acetonitrile/water, making it more nonpolar leading to the appearance of peptides with hydrophobic characteristics. These peptides are generally associated with biological activities, such antioxidant (Carrasco-Castilla et al., 2012), antihypertensive properties (Pripp et al., 2004), among others. These results are confirmed with studies that use yeast hydrolysates for production of antioxidant extracts (Jung et al., 2011) and antihypertensive effect (Mirzaei et al., 2015). In this context and in accordance with published studies aforementioned, relating hydrophilic/hydrophobic peptides with biological activities, <3 kDa fractions may have regulatory functions in the human system beyond nutritional richness, which will be further tested.

4. Conclusions

This study allowed us to develop, for the first time, a selective membrane filtration pilot process for recovering spent brewer's yeast fractions obtained by autolysis and enzymatic hydrolysis. Four added-value fractions with different sizes and molecular weights were obtained. The physicochemical characterization of each fraction, showed the nutritional value of all fractions relating to protein, mineral and carbohydrates. This process could be applied as an effective approach to nutrient regeneration/production, which can be used as supplements with biological properties in food and feed. They can also be used in the food industry for technological purposes due to their mineral and amino acids content.

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